11-1999

Temporal changes in allele frequencies in two reciprocally selected maize populations

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Theor Appl Genet (1999) 99:1166–1178

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Received: 10 June 1998 / Accepted: 29 April 1999

Abstract The effects of breeding on allele frequency changes at 82 restriction fragment length polymorphism (RFLP) loci were examined in two maize (*Zea mays* L.) populations undergoing reciprocal recurrent selection, Iowa Stiff Stalk Synthetic and Iowa Corn Borer Synthetic #1. After 12 cycles of selection, approximately 30% of the alleles were extinct and 10% near fixation in each population. A test of selective neutrality identified several loci in each population whose allele frequency changes cannot be explained by genetic drift; interpopulation mean expected heterozygosity increased for that subset of 28 loci but not for the remaining 54 loci. Mean expected heterozygosity within the two subpopulations decreased 39%, while the between-population component of genetic variation increased from 0.5% to 33.4% of the total. Effective population size is a key parameter for discerning allele frequency changes due to genetic drift versus those resulting from selection and genetic hitchhiking. Empirical estimates of effective population size for each population were within the range predicted by the breeding method.

Key words Effective population size · Selection · Genetic drift · Diversity · Restriction fragment length polymorphism

Introduction

Heterosis is the basis of the modern cultivars utilized in maize. The primary aim of maize breeders is to develop populations and inbred lines that can be crossed to form superior hybrids. Several widely used elite lines have been derived from populations improved by recurrent selection. Recurrent selection employs genetically broad-based populations and gradually increases the frequency of favorable alleles (reviewed by Hallauer 1985). A large body of empirically derived knowledge has accumulated on quantitative genetic and phenotypic responses to various types of recurrent selection (Hallauer and Miranda 1988, chapter 7); however, variation in response within and among selection programs indicates a limit to our understanding of the genome's response to conscious selection. Because a single cycle of recurrent selection may take 2–3 years to complete, recurrent selection programs necessitate long-term goals and investments of resources. An understanding of genetic components underlying the selection response is needed to aid in the design of effective and efficient recurrent selection breeding programs. Molecular markers have been used in several studies to examine genetic changes in maize populations undergoing selection (Heredia-Díaz et al. 1996 and references therein). Stuber et al. (1980) found allele frequency changes at eight out of eight isozyme loci to be associated with selection for yield in maize in four different long-term programs.

Both intra- and inter-population methods of recurrent selection are currently practiced. Comstock et al. (1949) originally proposed interpopulation recurrent selection. The objective of this method, termed reciprocal recurrent selection (RRS), is to improve the performance of an interpopulation cross of two genetically divergent populations. RRS involves the development of progenies within populations (e.g., S_1 lines, first-generation progenies from self-fertilized individuals), reciprocal crosses of progenies between populations, phenotypic evaluation of these testcrosses, and selection of progenies based on testcross results. Selected progenies are then intermated within each population to initiate the next cycle of selection (Fig. 1). RRS is designed to allow for genetic recombination within populations to maintain quantitative genetic variation, while minimizing inbreeding. It has

Communicated by A.L. Kahler

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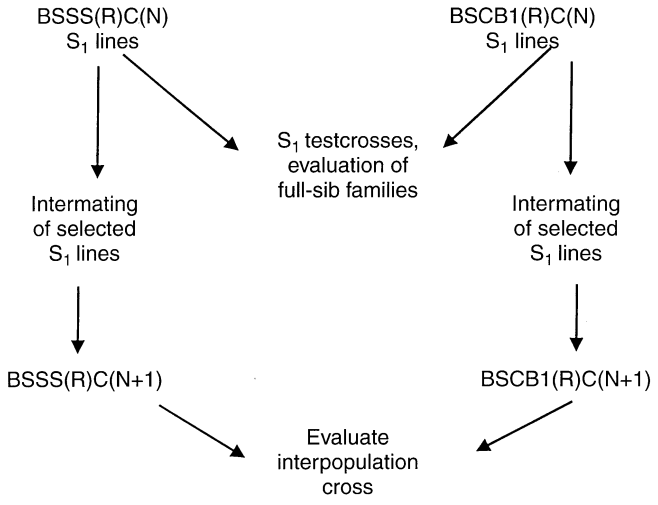

Fig. 1 A single cycle of the reciprocal recurrent selection breeding method. One-hundred plants from populations of approximately 500 individuals are self-fertilized. The S_1 progeny are evaluated in reciprocal testcrosses between populations, and 10–20 superior lines are selected per population. These are intermated within populations to form starting populations for the next cycle

been found to be especially effective for selection on quantitative traits of low heritability (Hallauer 1985).

Effective population size (N_e) is a parameter of critical importance in populations undergoing phenotypic selection, as it is related to the intensity of selection that can be effectively applied. There is some uncertainty concerning the theoretically expected N_e under RRS. A common assumption is that it equals the mean of N, the number of selected S_1 lines each cycle. It is also possible that N_e=2N–1, because the variance in the number of gametes furnished by parents to the next generation under this selection scheme is zero. It is not known to what degree (typically intense) selection influences N_e in this breeding scheme.

Two maize populations, Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic #1 (BSCB1), are currently in their 14th cycle of RRS in the Cooperative Federal-State maize breeding program at Iowa State University. Increased grain yield has been the primary target of selection, with reduced grain moisture at harvest and increased resistance to root and stalk lodging (lodging is when a plant falls towards a horizontal position) as secondarily selected traits. Selection has been highly successful; mean grain yield of the interpopulation cross improved 77% by cycle 11, relative to cycle 0, with concurrent favorable responses in the other traits (Keeratinijakal and Lamkey 1993a).

In a previous report we focused on the contributions of inbred progenitor lines to synthetic BSSS(R) and BSCB1(R) Cycle 0 (C0) populations and descriptions of genetic variation within and divergence between progenitor, C0 and C12 BSSS(R) and BSCB1(R) populations with genotypic data for 82 (RFLP) loci (Labate et al. 1997). In the study presented here, we used the same data set to look more closely at changes in allele frequencies within BSSS(R) and BSCB1(R). Our objectives were to (1) test the null hypothesis of genetic drift, i.e. changes in allele frequency at a particular locus within a population can be explained as resulting from the random sampling of gametes each generation; (2) examine genetic diversity for the two populations from the progenitors to C12 in terms of mean allele frequencies (Nei and Chesser 1983) and partitioning of diversity components (Chakraborty 1980); (3) compare expected heterozygosity of the interpopulation cross for C0 and C12; (4) obtain empirical estimates of effective population size (Waples 1989b) in BSSS(R) and BSCB1(R).

Materials and methods

Populations

The BSSS and BSCB1 populations have been undergoing RRS since 1949 (Penny and Eberhart 1971). Our study examines samples from three populations within BSSS(R) and BSCB1(R) representing three different stages in their selective history. BSSS(R) and BSCB1(R) were originally developed through a series of single, double or three-way and double-double crosses tracing back to 16 and 12 inbred lines, respectively (Fig. 2). The collections of inbred lines are herein referred to as progenitor (P) populations. Two of the BSSS progenitor inbred lines (CI617 and F1B1) were not available (lost); however, the two inbred parental lines of F1B1 (Fe and IndB2) have been included in our study. Progenitor lines have been maintained over several decades through self-pollination. The Cycle 0 (C0) populations were formed by random-mating the bulked seed from double-double crosses for five to six generations. These BSSS(R) and BSCB1(R) C0 populations were the starting material for RRS. Germplasm of both C0 populations has been maintained since 1949 through periodically planting and randomly mating several hundred plants. The total number of random mating generations and the absolute population sizes are not known. Samples of C0 used in our study represent individual plants from these populations. The C12 populations were originally synthesized by intermating 20 S_1 lines selected from C11. The resulting population was random-mated in 1989, and approximately 350 ears were harvested from both BSSS and BSCB1. Seed from these ears was bulked and planted, and mature plants were random-mated in 1990; approximately 350 ears were harvested from each. This bulked seed was used to grow BSSS(R) and BSCB1(R) C12 plants for our study.

Sampling of populations and RFLP analysis

Approximately 200 plants from each C0 and C12 were grown in 1992 in Ames, Iowa at low plant densities (4,050 plants/hectare); all individuals were labeled. Progenitors grown under similar conditions were sampled that year from Dr. A.R. Hallauer's nursery. Mature leaf tissue was collected, freeze-dried, and later ground and stored at –20°C. Leaf samples representing 100 individuals from each C0 and C12 population were chosen at random for genotyping. DNA isolation and RFLP analyses were performed according to the protocols described by Veldboom et al. (1994). Probe names are found in Tables 1 and 2. The loci on each chromosome are sorted according to map position based on information obtained from the Maize Genome Database (http://www.agron.missouri.edu) and from published maps (Davis et al. 1996; Matz et al. 1994). Because the loci were not mapped in BSSS and BSCB1 populations, map information must be regarded as approximate. Restriction enzyme *Hin*dIII was used for DNA digestion with all probes except *bnl5.62*, *umc76*, *umc140*, *umc26*, *umc60*, *npi398* and *php6005*, for which *Eco*RI was used. Details of scoring procedures can be found in Labate et al. (1997). Out of 100

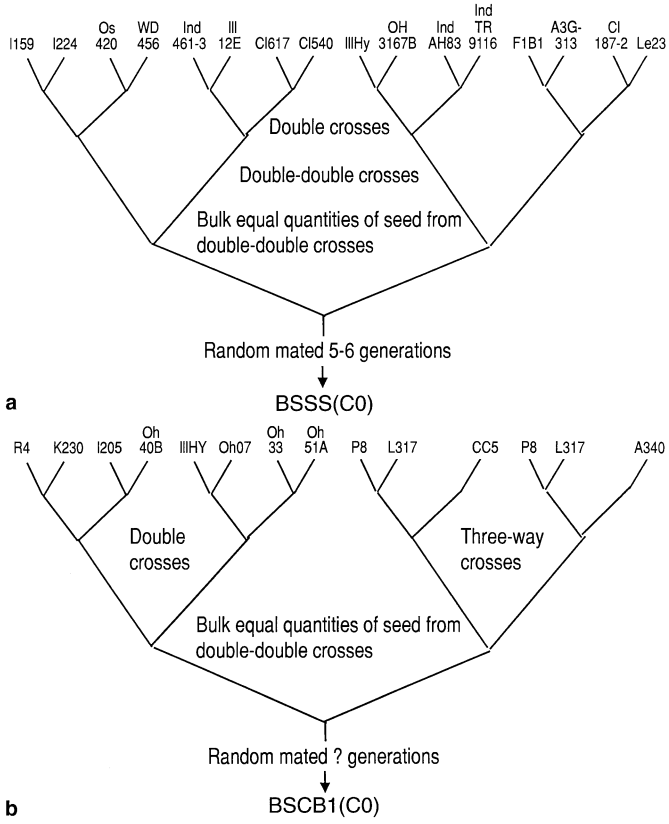

Fig. 2a, b Crosses used to construct BSSS(C0) (**a**) and BSCB1(C0) (**b**) from 16 and 12 inbred lines, respectively

nuclear genomic probes originally scored, 18 were eliminated from further analyses because their banding patterns were too complex to interpret. After these were eliminated, each RFLP probe was considered to detect a single locus, and variants at each locus were assumed to be allelic. Sample sizes of n=70–100 were obtained for each locus in BSSS(R) and BSCB1(R) C0 and C12 populations (mean=97.52), n=14–16 for BSSS(R) progenitors (mean=15.96) and n=11–12 for BSCB1(R) progenitors (mean=11.98).

Diversity partitioning

The software program GENESTRUT (Constantine et al. 1994) was used to measure genetic diversity with diploid genotypic data from the six populations, BSSS(R) and BSCB1(R) P, C0, and C12. 'Total' populations were defined as pooled BSSS(R) and BSCB1(R) populations of the same cycle or pooled progenitor populations. GENESTRUT calculates unbiased estimates of heterozygosity by using the method of Nei and Chesser (1983). Given s subpopulations, n_i individuals randomly sampled from the i^{th} subpopulation, and r alleles, observed heterozygosity is estimated as

$$\hat{H}_O = 1 - \sum_i^s \sum_k^r X_{ikk} / s$$

where X_{ikk} equals the observed frequency of genotype A_kA_k in the i^{th} subpopulation. H_S and H_T represent the expected heterozygosities under Hardy-Weinberg equilibrium or gene diversities, within subpopulations and in the total population, respectively, and are estimated as

$$\hat{H}_S = \frac{\tilde{n}}{\tilde{n}-1}\left(1 - \sum_k^r \overline{x_k^2} - \frac{\hat{H}_O}{2\tilde{n}}\right) \qquad \text{(sub)}$$

$$\hat{H}_T = 1 - \sum_k^r \bar{x}_k^2 + \hat{H}_S/(\tilde{n}s) - \hat{H}_O/(2\tilde{n}s) \qquad \text{(total)}$$

where

$$\overline{x_k^2} \equiv \left(\sum_i^s \sum_k^r x_{ik}^2\right)/s,$$

$$\bar{x}_k^2 \equiv \left(\sum_i^s x_{ik}/s\right)^2,$$

x_{ik} is the frequency of allele A_k in the sample from the i^{th} subpopulation, and $\tilde{n}$ equals the harmonic mean of $n_{i.}$. H_T can be partitioned into a component because of variation within populations, H_S, and between populations, D_{12}, by

$$H_T = H_S + D_{12}$$

(Chakraborty 1980).

Neutrality test

Waples (1989a) proposed a single-locus test of temporal variation in allele frequency. It tests whether the observed variation in allele frequency between two samples taken at different times can be explained as a sample drawn from a population of size N_e that has undergone t generations of genetic drift. The test statistic is distributed as a chi-square (Waples 1989a) and is calculated as

$$\chi^2 = \frac{(x-y)^2}{\mathrm{var}(x-y)}$$

where x equals the estimated allele frequency in an initial sample, y equals the estimated allele frequency in a subsequent sample and var(x-y) equals the variance of this difference. The derivation of the variance in (x-y) is explained in detail by Waples (1989a) and depends on the method of sampling, sample sizes, the number of generations that have passed, N_e and census size (total number of individuals in the population). We assumed that each cycle of selection is equivalent to a single generation and that effective size is equal to the harmonic mean of twice the number of selected lines minus one ($2N$–1). The additional generation during each cycle was ignored because it involves random-mated populations of 300–500 individuals, within which drift will be relatively small in comparison to the bottleneck generation of 10–20 selected S_1 lines. Under Plan II of Waples' (1989a) model it is necessary for census size to be at least twice the effective population size. We assume that the census size of these populations is about an order of magnitude larger than the effective population size, because only a small fraction of the population is selected to advance to the subsequent generation. The genotyped individuals were not part of the reproducing population, therefore, samples and N_e were mutually exclusive. Each locus was tested using a generalized procedure that accommodates multiple alleles and accounts for covariances of frequencies for different alleles sampled at different times (Waples 1989a, appendix).

Heterozygosity

Sample allele frequencies in C0 or C12 populations were used to estimate expected heterozygosities resulting from hypothetical crosses, assuming the probability of homozygosity of a given allele in an F_1 generation equaled the product of its frequencies in two crossed populations. Estimates of expected heterozygosity in F_1 with a hypothetical sample size of n=100 were obtained for various crosses with the unbiased estimator of Nei (1987, p 178), for locus l as

$$\hat{D}_l = 2n\left(1 - \sum \hat{x}_i^2\right)/(2n-1)$$

for n individuals, where $\hat{x}_i$ is the estimated frequency of the i^{th} allele:

$$\hat{x}_i = \tilde{x}_{ii} + \sum_{i \neq j} \tilde{x}_{ij}/2,$$

$\tilde{x}_{ii}$ equals the observed frequency of homozygotes and $\tilde{x}_{ij}$ equals the observed frequency of heterozygotes. Total gene diversity (Nei 1987, p 179) was calculated as

Table 1 RFLP probes, chromosomal locations and estimated allele frequencies for 82 loci in BSSS(R) populations[a]

Chromosome,	Probe	Progenitors										Cycle 0										Cycle 12									
		Allele										Allele										Allele									
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	*bnl5.62*	0.13	0.13	0.25	0.50							0.14	0.00	0.00	0.86							0.01	0.00	0.01	0.99						
	umc157	0.13	0.31	0.13	0.00	0.38	0.06					0.31	0.00	0.13	0.00	0.56	0.00					0.00	0.01	0.00	0.00	0.99	0.00				
	umc76	0.19	0.47	0.28	0.06							0.38	0.01	0.62	0.00							0.54	0.00	0.44	0.03						
	bnl10.38	0.19	0.13	0.50	0.06	0.13						0.00	0.00	0.69	0.17	0.15						0.01	0.00	0.72	0.24	0.03					
	umc167	0.88	0.06	0.06								0.52	0.37	0.12								0.02	0.00	0.99							
	umc128	0.09	0.66	0.13	0.13	0.00	0.00					0.35	0.35	0.06	0.12	0.00	0.12					0.07	0.01	0.00	0.00	0.00	0.93				
	umc140	0.06	0.66	0.16	0.13							0.00	0.45	0.02	0.53							0.00	0.55	0.01	0.45						
	umc84	0.63	0.06	0.00	0.13	0.06	0.13					0.67	0.00	0.13	0.06	0.07	0.07					0.01	0.00	0.31	0.24	0.06	0.39				
	bnl6.32	0.19	0.38	0.13	0.06	0.19	0.06					0.19	0.57	0.11	0.00	0.14	0.00					0.00	0.45	0.02	0.00	0.52	0.01				
2	*umc61*	0.19	0.44	0.19	0.19							0.52	0.48	0.00	0.00							0.13	0.87	0.00	0.00						
	bnl12.09	0.38	0.00	0.38	0.25							0.06	0.01	0.35	0.59							0.22	0.01	0.45	0.33						
	umc34	0.20	0.20	0.33	0.00	0.27						0.02	0.31	0.56	0.01	0.11						0.86	0.01	0.12	0.00	0.01					
	umc135	0.06	0.38	0.00	0.38	0.06	0.06	0.06				0.12	0.09	0.01	0.78	0.00	0.00	0.00				0.00	0.00	0.00	1.00	0.00	0.00	0.00			
	umc131	0.00	0.63	0.25	0.13							0.00	0.24	0.57	0.20							0.00	0.23	0.38	0.39						
	umc36	0.19	0.00	0.31	0.06	0.44						0.20	0.01	0.68	0.00	0.12						0.60	0.00	0.40	0.00	0.01					
	umc4	0.38	0.56	0.06								0.20	0.80	0.00								0.14	0.86	0.00							
	umc137	0.31	0.13	0.38	0.19							0.47	0.00	0.00	0.53							0.24	0.00	0.00	0.77						
3	*bnl8.35*	0.06	0.38	0.06	0.06	0.38	0.06					0.00	0.56	0.10	0.00	0.34	0.00					0.00	0.81	0.05	0.00	0.14	0.00				
	umc26	0.56	0.00	0.06	0.06	0.31	0.00					0.93	0.02	0.00	0.00	0.06	0.00					0.99	0.01	0.00	0.00	0.00	0.00				
	bnl5.14	0.06	0.94	0.00	0.00							0.22	0.78	0.00	0.00							0.00	1.00	0.00	0.00						
	umc60	0.94	0.06	0.00								1.00	0.00	0.00								1.00	0.00	0.01							
	bnl7.26	0.94	0.06									0.57	0.43									0.18	0.83								
4	*umc87*	0.81	0.00	0.19								0.54	0.00	0.46								0.48	0.00	0.53							
	umc31	0.81	0.00	0.19								0.53	0.00	0.47								0.44	0.00	0.56							
	bnl5.46	0.63	0.38									0.56	0.45									0.93	0.08								
	umc158	0.00	0.38	0.13	0.38	0.13						0.00	0.49	0.27	0.05	0.19						0.00	0.77	0.03	0.01	0.20					
	umc156	0.06	0.50	0.13	0.31							0.02	0.64	0.19	0.16							0.01	0.97	0.00	0.03						
	umc42	0.63	0.38									0.36	0.65									0.99	0.02								
	umc19	0.06	0.50	0.38	0.06							0.00	0.62	0.38	0.01							0.00	0.37	0.64	0.00						
	bnl5.67	0.94	0.06									0.68	0.32									0.15	0.85								
	umc15	0.13	0.06	0.69	0.13	0.00						0.32	0.00	0.62	0.05	0.02						0.01	0.00	0.99	0.00	0.01					
	bnl15.07	0.38	0.13	0.50								0.25	0.08	0.67								0.12	0.00	0.89							
5	*bnl8.33*	0.25	0.44	0.25	0.06							0.08	0.80	0.11	0.01							0.00	0.77	0.22	0.01						
	bnl6.25	0.25	0.25	0.00	0.38	0.13	0.00					0.01	0.23	0.00	0.60	0.15	0.02					0.00	0.00	0.00	1.00	0.01	0.00				
	umc147	0.38	0.25	0.13	0.13	0.13						0.47	0.31	0.10	0.12	0.00						0.39	0.61	0.00	0.01	0.00					
	bnl7.56	0.31	0.00	0.13	0.06	0.25	0.13	0.13				0.12	0.00	0.00	0.00	0.00	0.09	0.79				0.49	0.00	0.01	0.00	0.00	0.00	0.50			
	bnl7.71	0.63	0.19	0.13	0.06							0.57	0.10	0.00	0.33							0.20	0.38	0.00	0.41						
	bnl5.71	0.31	0.38	0.00	0.00	0.31						0.70	0.22	0.00	0.00	0.08						0.99	0.01	0.00	0.00	0.01					
	umc43	0.19	0.75	0.06								0.00	1.00	0.00								0.01	1.00	0.00							
	umc54	0.13	0.13	0.75								0.11	0.14	0.75								0.03	0.71	0.27							
	umc108	0.19	0.09	0.06	0.31	0.13	0.06	0.16				0.09	0.03	0.08	0.27	0.13	0.23	0.17				0.02	0.72	0.04	0.00	0.00	0.00	0.22			

Table 1 Continued

Chromosome,	Probe	Progenitors										Cycle 0										Cycle 12									
		Allele										Allele										Allele									
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
6	*umc85*	0.06	0.38	0.19	0.38							0.00	0.42	0.05	0.54							0.00	0.01	0.00	1.00						
	umc59	0.00	0.50	0.00	0.06	0.00	0.06	0.31	0.00	0.06		0.20	0.62	0.00	0.16	0.00	0.00	0.03	0.00	0.00		0.00	0.76	0.00	0.24	0.00	0.01	0.00	0.00	0.00	
	php20854	0.31	0.06	0.19	0.19	0.06	0.19	0.00				0.32	0.00	0.11	0.41	0.07	0.10	0.00				0.59	0.00	0.29	0.10	0.01	0.01	0.00			
	umc113B	0.06	0.09	0.06	0.38	0.22	0.19					0.00	0.00	0.13	0.49	0.00	0.37					0.00	0.00	0.24	0.36	0.00	0.40				
	umc21	0.50	0.00	0.31	0.19							0.74	0.00	0.10	0.17							0.71	0.00	0.00	0.29						
	bnl3.03	0.44	0.06	0.19	0.31							0.29	0.17	0.26	0.29							0.00	0.14	0.40	0.47						
	umc46	0.06	0.25	0.56	0.13							0.11	0.33	0.56	0.01							0.32	0.26	0.42	0.00						
	bnl5.47	0.00	0.13	0.81	0.06							0.00	0.00	0.78	0.22							0.00	0.00	0.43	0.57						
	umc38	0.00	0.50	0.06	0.44	0.00						0.00	0.70	0.03	0.25	0.02						0.00	0.01	0.00	0.99	0.00					
	php10016	0.50	0.06	0.13	0.31							0.42	0.04	0.02	0.53							0.03	0.01	0.00	0.96						
	umc62	0.88	0.06	0.06								0.51	0.49	0.00								0.71	0.29	0.00							
	umc134	0.44	0.25	0.31								0.41	0.38	0.22								1.00	0.00	0.00							
7	*bnl15.40*	0.25	0.13	0.00	0.50	0.13						0.56	0.00	0.00	0.32	0.12						0.01	0.54	0.00	0.00	0.45					
	umc110	0.50	0.06	0.13	0.06	0.06	0.13	0.06	0.00			0.19	0.14	0.22	0.00	0.00	0.46	0.01	0.00			0.00	0.00	0.00	0.00	0.00	0.99	0.02	0.00		
	php20746	0.00	0.06	0.19	0.44	0.19	0.00	0.06	0.06			0.01	0.00	0.07	0.61	0.25	0.00	0.07	0.00			0.00	0.00	0.24	0.42	0.34	0.00	0.00	0.00		
	bnl8.32	0.00	0.13	0.06	0.06	0.31	0.25	0.19				0.00	0.47	0.00	0.00	0.24	0.07	0.22				0.00	0.39	0.00	0.00	0.01	0.00	0.60			
	bnl14.07	0.00	0.07	0.07	0.36	0.00	0.00	0.00	0.50			0.00	0.00	0.00	0.69	0.00	0.00	0.02	0.29			0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.47		
	npi398	0.06	0.75	0.19	0.00							0.00	0.60	0.40	0.01							0.01	0.56	0.43	0.01						
	umc35	0.38	0.19	0.13	0.25	0.00	0.00	0.06				0.41	0.48	0.01	0.10	0.01	0.00	0.00				0.60	0.00	0.00	0.41	0.00	0.00	0.00			
8	*bnl13.05*	0.00	0.06	0.00	0.13	0.00	0.19	0.25	0.25	0.13		0.00	0.47	0.01	0.00	0.00	0.43	0.06	0.00	0.04		0.00	0.49	0.00	0.00	0.00	0.33	0.01	0.00	0.16	
	bnl9.11	0.66	0.09	0.25								0.99	0.00	0.01								1.00	0.00	0.00							
	bnl9.44	1.00	0.00									0.93	0.07									0.23	0.78								
	bnl10.39	0.13	0.34	0.22	0.31							0.00	0.76	0.02	0.22							0.00	0.55	0.01	0.45						
	umc120	0.22	0.78									0.59	0.42									0.16	0.84								
	umc89	0.25	0.19	0.38	0.06	0.13						0.09	0.08	0.59	0.00	0.25						0.00	0.04	0.44	0.00	0.52					
	umc30	0.00	0.06	0.25	0.19	0.13	0.06	0.00	0.13	0.13	0.06	0.00	0.53	0.20	0.02	0.00	0.00	0.02	0.16	0.09	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.79	0.01	0.00
9	*c1*	0.06	0.00	0.81	0.13							0.09	0.00	0.82	0.08							0.00	0.00	0.66	0.34						
	umc113A	0.06	0.31	0.19	0.06	0.06	0.31					0.02	0.45	0.07	0.09	0.12	0.26					0.01	0.65	0.00	0.34	0.01	0.00				
	bnl3.06	0.88	0.00	0.06	0.06							1.00	0.01	0.00	0.00							0.99	0.01	0.00	0.00						
	umc81	0.44	0.13	0.38	0.06							0.03	0.15	0.49	0.33							0.01	0.00	0.99	0.00						
	bnl5.10	0.06	0.31	0.63								0.11	0.03	0.86								0.22	0.01	0.78							
	umc153	0.06	0.06	0.19	0.63	0.06						0.27	0.00	0.08	0.56	0.10						0.42	0.00	0.01	0.57	0.00					
	umc95	0.69	0.06	0.06	0.19							0.56	0.03	0.10	0.31							0.50	0.02	0.39	0.09						
	bnl5.09	0.00	0.19	0.56	0.25	0.00						0.00	0.33	0.49	0.17	0.03						0.01	0.60	0.39	0.01	0.00					
10	*bnl3.04*	0.00	0.06	0.31	0.56	0.06	0.00					0.00	0.30	0.02	0.69	0.00	0.00					0.00	0.49	0.01	0.51	0.00	0.00				
	php6005	0.13	0.53	0.09	0.19	0.06						0.16	0.40	0.08	0.37	0.00						0.01	0.65	0.02	0.33	0.00					
	umc155	0.00	0.31	0.56	0.13							0.00	0.03	0.91	0.07							0.00	0.01	1.00	0.00						
	umc159	0.50	0.50	0.00								0.20	0.81	0.00								0.33	0.68	0.00							
	umc57	0.13	0.44	0.38	0.06							0.19	0.40	0.33	0.09							0.02	0.50	0.05	0.44						
	bnl10.13	0.00	0.06	0.25	0.13	0.06	0.06	0.00	0.25	0.06	0.13	0.00	0.00	0.42	0.01	0.00	0.00	0.00	0.27	0.00	0.30	0.00	0.00	0.51	0.05	0.00	0.00	0.04	0.40	0.00	0.02
	bnl7.49	0.19	0.06	0.25	0.38	0.06	0.00	0.06				0.00	0.16	0.13	0.71	0.00	0.00	0.00				0.00	0.00	0.02	0.98	0.01	0.00	0.00			

[a] Because of rounding error, allele frequencies may not sum to one

Table 2 RFLP probes, chromosomal locations and estimated allele frequencies for 82 loci in BSCB1(R) populations[a]

Chromosome,	Probe	Progenitors										Cycle 0										Cycle 12									
		Allele										Allele										Allele									
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	*bnl5.62*	0.17	0.04	0.17	0.63							0.15	0.13	0.20	0.52							0.00	0.01	0.94	0.05						
	umc157	0.08	0.25	0.25	0.17	0.17	0.08					0.16	0.34	0.09	0.11	0.22	0.08					0.00	0.98	0.00	0.02	0.01	0.00				
	umc76	0.08	0.25	0.42	0.25							0.19	0.43	0.23	0.15							0.04	0.18	0.77	0.02						
	bnl10.38	0.29	0.46	0.17	0.00	0.08						0.23	0.41	0.25	0.00	0.11						0.25	0.73	0.03	0.00	0.00					
	umc167	0.92	0.08	0.00								1.00	0.01	0.00								1.00	0.00	0.00							
	umc128	0.00	0.42	0.25	0.25	0.08	0.00					0.00	0.31	0.36	0.15	0.14	0.04					0.00	0.22	0.30	0.01	0.47	0.00				
	umc140	0.08	0.50	0.25	0.17							0.02	0.53	0.39	0.06							0.00	0.93	0.00	0.07						
	umc84	0.58	0.08	0.08	0.08	0.08	0.08					0.49	0.23	0.13	0.08	0.06	0.03					0.74	0.00	0.00	0.00	0.00	0.27				
	bnl6.32	0.17	0.46	0.00	0.29	0.00	0.00					0.15	0.48	0.02	0.28	0.07	0.00					0.00	0.00	0.00	0.54	0.46	0.00				
2	*umc61*	0.29	0.46	0.08	0.17							0.48	0.35	0.06	0.10							0.76	0.24	0.00	0.00						
	bnl12.09	0.25	0.17	0.42	0.17							0.20	0.16	0.40	0.24							0.00	1.00	0.00	0.00						
	umc34	0.08	0.08	0.50	0.08	0.25						0.07	0.13	0.46	0.04	0.30						0.00	0.49	0.48	0.00	0.02					
	umc135	0.00	0.13	0.08	0.79	0.00	0.00	0.00				0.00	0.01	0.06	0.93	0.00	0.00	0.00				0.00	0.00	0.07	0.93	0.00	0.00	0.00			
	umc131	0.08	0.50	0.42	0.00							0.10	0.67	0.24	0.00							0.00	1.00	0.00	0.00						
	umc36	0.50	0.08	0.33	0.00	0.08						0.29	0.01	0.52	0.00	0.19						0.16	0.01	0.50	0.02	0.32					
	umc4	0.50	0.42	0.08								0.36	0.30	0.33								0.00	0.84	0.16							
	umc137	0.25	0.13	0.08	0.54							0.31	0.06	0.06	0.58							0.05	0.00	0.00	0.96						
3	*bnl8.35*	0.00	0.25	0.00	0.33	0.33	0.08					0.00	0.16	0.01	0.33	0.41	0.08					0.00	0.98	0.00	0.01	0.01	0.01				
	umc26	0.75	0.00	0.04	0.04	0.17	0.00					0.57	0.14	0.01	0.04	0.22	0.02					0.97	0.03	0.00	0.00	0.00	0.00				
	bnl5.14	0.08	0.67	0.17	0.08							0.08	0.84	0.04	0.04							0.00	1.00	0.01	0.00						
	umc60	0.75	0.17	0.08								0.81	0.07	0.12								0.79	0.01	0.21							
	bnl7.26	0.83	0.17									0.85	0.15									1.00	0.00								
4	*umc87*	0.67	0.08	0.25								0.41	0.33	0.26								0.73	0.01	0.26							
	umc31	0.42	0.33	0.25								0.36	0.37	0.27								0.75	0.00	0.26							
	bnl5.46	0.58	0.42									0.75	0.26									0.74	0.26								
	umc158	0.04	0.38	0.17	0.25	0.17						0.15	0.46	0.13	0.18	0.09						0.00	0.56	0.10	0.34	0.01					
	umc156	0.17	0.75	0.00	0.08							0.31	0.57	0.00	0.13							0.48	0.00	0.00	0.52						
	umc42	0.33	0.67									0.58	0.43									0.25	0.76								
	umc19	0.17	0.50	0.33	0.00							0.05	0.44	0.52	0.00							0.00	0.08	0.93	0.00						
	bnl5.67	0.92	0.08									0.97	0.03									1.00	0.00								
	umc15	0.08	0.00	0.71	0.13	0.08						0.13	0.00	0.59	0.23	0.06						0.02	0.00	0.00	0.21	0.77					
	bnl15.07	0.21	0.13	0.67								0.19	0.15	0.66								0.17	0.00	0.83							
5	*bnl8.33*	0.08	0.17	0.50	0.25							0.00	0.17	0.53	0.30							0.00	0.00	0.45	0.55						
	bnl6.25	0.18	0.18	0.05	0.23	0.27	0.09					0.15	0.15	0.07	0.24	0.26	0.13					0.01	0.03	0.00	0.00	0.62	0.35				
	umc147	0.50	0.17	0.00	0.25	0.08						0.33	0.04	0.00	0.59	0.03						0.95	0.00	0.00	0.06	0.00					
	bnl7.56	0.33	0.08	0.17	0.00	0.00	0.17	0.25				0.38	0.02	0.05	0.00	0.05	0.19	0.33				0.65	0.00	0.00	0.00	0.00	0.35	0.00			
	bnl7.71	0.54	0.25	0.13	0.08							0.67	0.19	0.10	0.04							0.00	1.00	0.00	0.00						
	bnl5.71	0.17	0.38	0.08	0.04	0.33						0.12	0.41	0.21	0.01	0.25						0.11	0.47	0.02	0.00	0.41					
	umc43	0.33	0.67	0.00								0.22	0.79	0.00								0.20	0.80	0.00							
	umc54	0.00	0.04	0.96								0.00	0.06	0.94								0.00	0.00	1.00							
	umc108	0.21	0.33	0.13	0.13	0.04	0.08	0.08				0.21	0.35	0.16	0.12	0.03	0.09	0.04				0.51	0.33	0.00	0.00	0.00	0.00	0.16			

Table 2 Continued

Chromosome, Probe		Progenitors										Cycle 0										Cycle 12									
		Allele										Allele										Allele									
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
6	*umc85*	0.63	0.17	0.00	0.21							0.46	0.03	0.00	0.52							0.00	0.06	0.00	0.95						
	umc59	0.08	0.38	0.04	0.25	0.08	0.08	0.00	0.08	0.00		0.18	0.39	0.00	0.11	0.03	0.12	0.00	0.18	0.00		0.00	0.21	0.00	0.32	0.00	0.46	0.00	0.02	0.00	
	php20854	0.00	0.00	0.17	0.29	0.08	0.21	0.25				0.00	0.00	0.18	0.22	0.03	0.44	0.12				0.00	0.00	0.21	0.42	0.00	0.37	0.01			
	umc113B	0.00	0.00	0.08	0.42	0.17	0.33					0.00	0.00	0.08	0.47	0.08	0.36					0.00	0.00	0.41	0.02	0.01	0.56				
	umc21	0.58	0.08	0.17	0.17							0.56	0.06	0.25	0.14							0.35	0.32	0.33	0.00						
	bnl3.03	0.33	0.00	0.17	0.50							0.44	0.00	0.25	0.32							0.27	0.00	0.61	0.12						
	umc46	0.25	0.08	0.63	0.04							0.31	0.19	0.42	0.08							0.00	0.53	0.45	0.03						
	bnl5.47	0.17	0.33	0.42	0.08							0.16	0.32	0.50	0.02							0.00	0.00	1.00	0.00						
	umc38	0.25	0.17	0.00	0.50	0.08						0.27	0.41	0.00	0.22	0.10						0.81	0.05	0.00	0.14	0.01					
	php10016	0.29	0.50	0.00	0.21							0.30	0.50	0.00	0.20							0.08	0.88	0.00	0.04						
	umc62	0.88	0.13	0.00								0.94	0.06	0.00								1.00	0.00	0.00							
	umc134	0.46	0.21	0.33								0.52	0.18	0.30								0.23	0.49	0.28							
7	*bnl15.40*	0.33	0.08	0.08	0.33	0.17						0.27	0.02	0.02	0.46	0.23						0.97	0.00	0.01	0.03	0.00					
	umc110	0.25	0.17	0.33	0.00	0.00	0.08	0.08	0.08			0.26	0.17	0.30	0.00	0.00	0.11	0.09	0.08			0.01	0.42	0.00	0.00	0.00	0.01	0.57	0.00		
	php20746	0.25	0.00	0.17	0.04	0.08	0.38	0.00	0.08			0.05	0.00	0.20	0.37	0.22	0.16	0.00	0.00			0.10	0.00	0.50	0.40	0.00	0.00	0.00	0.00		
	bnl8.32	0.08	0.17	0.17	0.08	0.25	0.08	0.17				0.10	0.06	0.00	0.13	0.37	0.13	0.22				0.05	0.00	0.00	0.01	0.87	0.04	0.02			
	bnl14.07	0.04	0.04	0.13	0.46	0.00	0.08	0.08	0.17			0.01	0.12	0.03	0.34	0.15	0.08	0.01	0.27			0.00	0.00	0.00	0.82	0.07	0.00	0.00	0.11		
	npi398	0.00	0.42	0.17	0.42							0.00	0.45	0.30	0.25							0.11	0.00	0.27	0.62						
	umc35	0.54	0.00	0.08	0.21	0.00	0.17	0.00				0.56	0.11	0.07	0.16	0.10	0.00	0.00				0.70	0.00	0.27	0.01	0.03	0.00	0.00			
8	*bnl13.05*	0.08	0.13	0.08	0.00	0.08	0.04	0.25	0.25	0.08		0.10	0.06	0.02	0.02	0.08	0.09	0.38	0.11	0.14		0.00	0.00	0.00	0.00	0.28	0.48	0.00	0.04	0.20	
	bnl9.11	0.71	0.08	0.21								0.74	0.01	0.25								0.70	0.01	0.29							
	bnl9.44	0.92	0.08									0.97	0.03									1.00	0.00								
	bnl10.39	0.00	0.67	0.25	0.08							0.00	0.54	0.08	0.38							0.00	0.18	0.83	0.00						
	umc120	0.25	0.75									0.20	0.81									0.00	1.00								
	umc89	0.00	0.13	0.54	0.17	0.17						0.02	0.02	0.77	0.03	0.16						0.00	0.00	1.00	0.00	0.00					
	umc30	0.08	0.17	0.42	0.00	0.00	0.00	0.13	0.00	0.13	0.08	0.08	0.09	0.23	0.00	0.00	0.04	0.14	0.06	0.26	0.10	0.00	0.00	0.00	0.00	0.00	0.03	0.28	0.00	0.69	0.00
9	*c1*	0.25	0.08	0.67	0.00							0.15	0.18	0.68	0.00							0.00	0.00	1.00	0.00						
	umc113A	0.08	0.17	0.21	0.17	0.00	0.38					0.11	0.16	0.15	0.21	0.00	0.38					0.22	0.08	0.13	0.16	0.00	0.41				
	bnl3.06	0.83	0.17	0.00	0.00							0.89	0.12	0.00	0.00							0.63	0.38	0.00	0.00						
	umc81	0.58	0.00	0.33	0.08							0.54	0.00	0.15	0.31							1.00	0.00	0.00	0.00						
	bnl5.10	0.00	0.46	0.54								0.13	0.53	0.34								0.00	0.41	0.59							
	umc153	0.00	0.08	0.17	0.63	0.13						0.00	0.02	0.37	0.54	0.06						0.00	0.00	0.87	0.13	0.00					
	umc95	0.33	0.13	0.29	0.25							0.39	0.07	0.19	0.35							0.18	0.00	0.82	0.00						
	bnl5.09	0.04	0.42	0.33	0.13	0.08						0.05	0.23	0.58	0.11	0.03						0.66	0.00	0.34	0.00	0.00					
10	*bnl3.04*	0.21	0.00	0.00	0.54	0.17	0.08					0.13	0.00	0.00	0.37	0.39	0.13					0.20	0.00	0.00	0.67	0.14	0.00				
	php6005	0.29	0.29	0.21	0.13	0.08						0.23	0.23	0.35	0.13	0.05						0.00	0.45	0.55	0.00	0.00					
	umc155	0.08	0.58	0.17	0.17							0.07	0.42	0.29	0.22							0.00	0.02	0.93	0.05						
	umc159	0.13	0.79	0.08								0.08	0.82	0.10								0.46	0.54	0.00							
	umc57	0.25	0.25	0.50	0.00							0.23	0.43	0.35	0.00							0.02	0.35	0.63	0.00						
	bnl10.13	0.08	0.00	0.25	0.29	0.00	0.00	0.08	0.08	0.04	0.17	0.06	0.00	0.42	0.22	0.00	0.00	0.07	0.02	0.00	0.23	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.01	0.00	0.86
	bnl7.49	0.00	0.17	0.29	0.17	0.08	0.04	0.25				0.00	0.17	0.25	0.33	0.06	0.00	0.19				0.00	0.00	0.00	1.00	0.00	0.00	0.00			

[a] Because of rounding error, allele frequencies may not sum to one

$\hat{D} = \sum_{l=1}^{m} \hat{D}_l / m$

for m loci, where $\hat{D}_l$ is the value of $\hat{D}$ for the l^{th} locus. Separate estimates were obtained for neutral and nonneutral sets of loci as identified by Waples' (1989a) test.

Effective population size

Effective population sizes and their confidence intervals were estimated for BSSS(R) and BSCB1(R) by Waples' (1989b) temporal method with C0 and C12 as generations 1 and 12, respectively. Three different estimators of $\hat{f}$, the standardized variance in allele frequency change, were compared. All three estimators are available in the literature, and we had no reason to prefer one. These were proposed by Nei and Tajima (1981):

$$\hat{f} = \frac{(x-y)^2}{(x+y)/2 - xy}$$

Pollak (1983):

$$\hat{f} = \frac{(x-y)^2}{(x+y)/2 - [(x+y)/2]^2}$$

and Krimbas and Tsakas (1971):

$$\hat{f} = \frac{(x-y)^2}{x(1-x)}.$$

For these estimators, the numerator represents the change in allele frequency between two samples taken at different times, and the denominator attempts to standardize the values among loci by correcting for initial differences in allele frequencies. f (mean of $\hat{f}$) was calculated for all loci, neutral loci and nonneutral loci [as identified by Waples' test (1989a)]. Alleles at initially high frequencies that reach fixation will upwardly bias the estimate of effective population size because they are limited to small changes in frequency. Therefore, loci with the frequency of the C0 common allele greater than 0.90 were excluded from these analyses. Effective population size was estimated based on Plan II sampling, which assumes that the samples and N_e are mutually exclusive and can be considered as independent binomial draws from the same initial gamete pool, using this formula:

$$\hat{N}_e = \frac{t}{2[\bar{f} - 1/(2S_0) - 1/(2S_t)]}$$

where t is time in generations (t=12), and S_0 and S_t equal the harmonic mean over loci of the number of diploid individuals sampled from each population at C0 and C12, respectively. The 95% confidence intervals for $\hat{N}_e$ were calculated based on the number of independent alleles, n.

$$95\%\ \text{CI for } \hat{N}_e = \left[\frac{n\bar{f}}{\chi^2_{0.025,n}}, \frac{n\bar{f}}{\chi^2_{0.975,n}} \right]$$

Results

Allele frequency distributions

The progenitor populations were characterized by a large proportion of alleles at low frequencies (Tables 1–3, Fig. 3). About 30% of the total number of alleles in BSSS(R)P and BSCB1(R)P were at frequencies of 0.10 or less. The C0 populations should have similar allele frequencies to progenitor populations because there were five to six generations of random-mating between P and C0 with population sizes of several hundred individuals. BSCB1(R)P and C0 populations contained similar numbers of alleles and a similar mean allele frequency, but

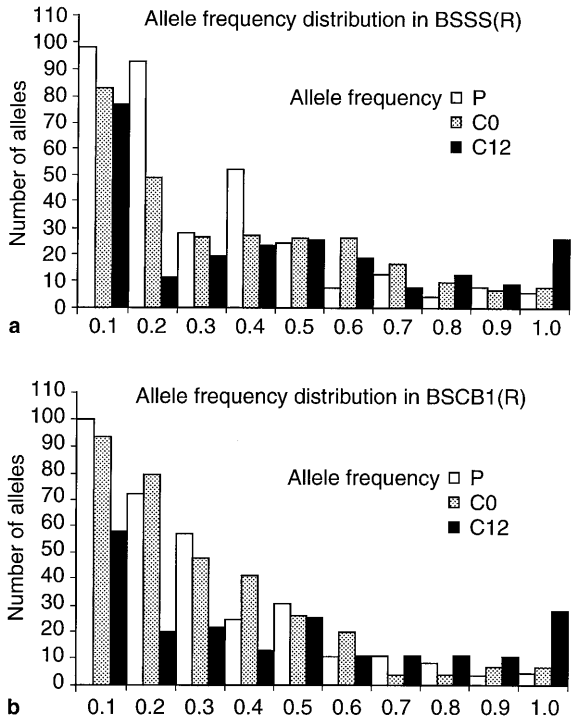

Fig. 3a, b Allele frequency versus number of alleles in BSSS(R) (**a**) and BSCB1(R) (**b**) P, C0 and C12 populations

Table 3 Number of alleles at 82 RFLP loci and their mean frequencies in BSSS(R) and BSCB1(R) populations

Population	Number of alleles	Mean allele frequency±SE
BSSS(R)P	330	0.29±0.014
BSSS(R)C0	275	0.34±0.016
BSSS(R)C12	225	0.41±0.019
BSCB1(R)P	325	0.29±0.012
BSCB1(R)C0	333	0.28±0.012
BSCB1(R)C12	207	0.49±0.029

BSSS(R) lost nearly 17% of its total number of alleles between BSSS(R)P and BSSS(R)C0. The increase in mean allele frequency between BSSS(R)P and BSSS(R)C0 suggests that primarily rare alleles were lost. These observations are consistent with previous results indicating that the BSSS(R)C0 population sampled for our study is not identical to the originally synthesized BSSS(R)C0 (Labate et al. 1997). We suspect that decades of population maintenance has led to the loss of alleles through drift. By C12 the frequency distributions became more U-shaped. Of the alleles 10% were near fixation (frequency of 0.9–1.0), and about 30% were near extinction (frequency of 0.10 or less). The mean allele frequency in BSCB1(R)C12 was significantly larger than the mean allele frequencies in BSSS(R)C12 (0.49±0.029 vs. 0.41±0.019), although they were very similar (0.29±0.012 vs. 0.29±0.014) in the progenitor populations. The shape of the allele frequency distribution seemed more uniform in the intermediate classes in

Table 4 Mean values of diversity in BSSS(R) and BSCB1(R) populations

	P	C0	C12
H_0[a]	0.029	0.484	0.303
H_S[b]	0.625	0.532	0.325
H_T[c]	0.628	0.576	0.489
D_{12}[d]	0.003	0.044	0.164

[a] Observed heterozygosity
[b] Subpopulation expected heterozygosity
[c] Total expected heterozygosity
[d] Between-population component of diversity

BSCB1(R)C12, and there were fewer rare alleles. The numbers of alleles approaching fixation were 25 and 28 in BSSS(R) and BSCB1(R) C12, respectively.

Genetic diversity

The mean observed heterozygosity across 82 RFLP loci in BSSS(R) and BSCB1(R) reveals a pattern ranging from the highly inbred progenitors (P) to the highly heterozygous C0 (Table 4). A large proportion (37%) of total observed heterozygosity was lost between C0 and C12; expected heterozygosity within subpopulations decreased 39%. A comparison of the observed heterozygosity and expected heterozygosity within subpopulations suggests a small amount of heterozygote deficiency, but most of the loci were in Hardy-Weinberg equilibrium within C0 and C12 subpopulations (data not shown). The expected subpopulation heterozygosity and expected total heterozygosity for the progenitor populations were very similar, indicating that most of the original genetic variation was found within both subpopulations rather than between them. The expected heterozygosity within subpopulations decreased substantially between P and C0, probably because of the loss of alleles in BSSS(R)C0. By C12 the expected total heterozygosity was relatively larger than expected heterozygosity within subpopulations, implying that the two populations became differentiated in their allelic compositions. The *between*-population component of genetic variation, D_{12}, was 0.5% of the total diversity in the progenitors, 7.7% in C0 and 33.4% by C12.

Neutrality tests

Allele frequencies are reported for each locus in BSSS(R) and BSCB1(R) (Table 1 and Table 2). Waples' (1989a) test was applied to each locus to detect if any of the observed changes in allele frequency between C0 and C12 within populations were greater than those expected by drift alone. The harmonic mean of the number of selected lines over 12 cycles equals 12 in these populations. Loci were tested for statistically significant changes in allele frequency assuming an effective population size of $2N$–1=23 using Waples' (1989a) model. There

Table 5 Waples' (1989a) test[a] for temporal changes in allele frequency between Cycle 0 and Cycle 12 for BSSS(R) and BSCB1(R) loci

Population	Locus	Chromosome	n_0[b]	n_t	χ^2	*df*
BSSS(R)	*umc167*	1	100	100	27.26***	2
	umc128	1	95	100	22.77***	4
	umc84	1	100	100	11.27*	4
	umc34	2	100	99	81.90***	4
	umc42	4	100	100	7.09**	1
	bnl5.67	4	99	99	5.39*	1
	umc54	5	98	90	10.50**	2
	umc108	5	97	98	46.73***	6
	umc38	6	100	100	11.67**	3
	umc134	6	100	100	6.05*	2
	bnl15.40	7	99	99	114.56***	3
	bnl9.44	8	99	100	26.36***	1
	umc30	8	99	100	11.93*	5
	bnl10.13	10	99	100	10.46*	4
BSCB1(R)	*bnl5.62*	1	95	96	13.51***	3
	bnl6.32	1	82	81	11.89*	4
	bnl12.09	2	97	98	19.38***	3
	bnl8.35	3	89	95	18.53***	4
	umc156	4	100	100	7.39*	2
	umc15	4	100	100	30.90***	3
	bnl7.71	5	96	100	16.81***	3
	bnl15.40	7	99	100	9.73*	4
	umc110	7	100	99	14.13**	5
	npi398	7	97	95	25.62***	3
	bnl10.39	8	98	100	26.22***	2
	umc95	9	93	99	10.40*	3
	bnl5.09	9	96	99	25.61***	4
	umc155	10	99	99	8.14*	3
	umc159	10	100	99	7.63*	2

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$
Only loci with significant values of the test statistic are listed. Names shown in boldface were significant in both populations. At locus *bnl15.40*, a different allele reached high frequency in each Cycle 12 population
[a] Based on N_e=23, see Tables 1 and 2 for allele frequency data
[b] n_0 equals sample size at Cycle 0, n_t equals sample size at Cycle 12

Table 6 Expected heterozygosity of population crosses for subsets of loci, means and their standard error for n=100 individuals

Population with significant Walples' tests	Loci	Cross			
		BSSS(R)C0× BSCB1(R)C0		BSSS(R)C12× BSCB1(R)C12	
		Mean	SE	Mean	SE
BSSS(R)	Neutral (n=68)	0.628	0.0207	0.609	0.0363
	Nonneutral (n=14)	0.602	0.0629	0.879	0.0293
BSCB1(R)	Neutral (n=67)	0.622	0.0237	0.633	0.0355
	Nonneutral (n=15)	0.629	0.0306	0.754	0.0774
Pooled	Neutral (n=54)	0.628	0.0248	0.576	0.0397
	Nonneutral (n=28)	0.614	0.0349	0.808	0.0444

Table 7 Estimates of effective population size and 95% confidence intervals with different estimators of the standardized variance in allele frequency change, $\hat{f}$

	$\hat{f}$, Nei and Tajima (1981)		$\hat{f}$, Pollak (1983)		$\hat{f}$, Krimbas and Tsakas (1971)	
	BSSS(R)	BSCB1(R)	BSSS(R)	BSCB1(R)	BSSS(R)	BSCB1(R)
All loci[a]						
$\bar{f}$[b]	0.29	0.33	0.31	0.34	0.58	0.47
(S_0, S_{12})[c]	(98.0, 97.6)	(96.3, 96.8)	(98.0, 97.6)	(96.3, 96.8)	(98.0, 97.6)	(96.3, 96.8)
N_e	21	19	20	18	11	13
95% C.I.	[17, 26]	[16, 22]	[16, 24]	[15, 22]	[9, 13]	[11, 16]
Neutral loci[d]						
$\bar{f}$	0.22	0.27	0.21	0.27	0.24	0.29
(S_0, S_{12})	(97.8, 97.0)	(96.4, 96.8)	(97.8, 97.0)	(96.4, 96.8)	(97.8, 97.0)	(96.4, 96.8)
N_e	29	23	30	23	26	22
95% C.I.	[22, 36]	[19, 28]	[24, 38]	[19, 28]	[20, 33]	[17, 26]
Nonneutral loci[c]						
$\bar{f}$	0.63	0.58	0.71	0.65	2.14	1.22
(S_0, S_{12})	(98.9, 98.9)	(95.8, 97.1)	(98.9, 98.9)	(95.8, 97.1)	(98.9, 98.9)	(95.8, 97.1)
N_e	10	11	9	9	3	5
95% C.I.	[6, 14]	[7, 15]	[5, 13]	[6, 14]	[2, 4]	[3, 7]

Loci with frequency of C0 common allele >0.90 were excluded from analyses of 'All loci' and 'Neutral loci'. For Krimbas and Tsakas'estimator, alleles with an estimated frequency of 0.0 at Cycle 0 were excluded from all analyses because they give an estimate of infinity for $\hat{f}$

[a] f was based on 198 and 246 independent alleles in BSSS(R) and BSCB1(R), respectively, for Nei and Tajima's and Pollak's estimators, and 186 and 244 independent alleles in BSSS(R) and BSCB1(R), respectively, for Krimbas and Tsakas' estimator

[b] Mean $\hat{f}$ over loci

[c] Sample sizes S_0 and S_{12} refer to the harmonic mean over loci of the number of diploid individuals sampled from each population at Cycle 0 and Cycle 12, respectively

[d] f was based on 157 and 198 independent alleles in BSSS(R) and BSCB1(R), respectively, for Nei and Tajima's and Pollak's estimators, and 147 and 197 independent alleles in BSSS(R) and BSCB1(R), respectively, for Krimbas and Tsakas' estimator

[e] f was based on 42 and 48 independent alleles for BSSS(R) and BSCB1(R), respectively, for Nei and Tajima's and Pollak's estimators, and 40 and 47 independent alleles in BSSS(R) and BSCB1(R), respectively, for Krimbas and Tsakas' estimator

were 14 nonneutral loci identified in BSSS(R), and 15 were identified in BSCB1(R) (Table 5). No additional significant tests would be obtained using any smaller value of N_e because such tests would be more conservative.

Expected heterozygosity of interpopulation crosses

Labate et al. (1997) measured mean expected heterozygosity of interpopulation crosses and found no significant difference between BSSS(R)C0×BSCB1(R)C0 and BSSS(R)C12×BSCB1(R)C12 for the 82 RFLP loci. We tested the hypothesis that the nonneutral loci (based on Table 5) increased in mean expected heterozygosity be-

tween C0 and C12 but that this effect was masked by the neutral loci. The results (Table 6) supported this hypothesis. The 28 nonneutral loci increased in mean expected heterozygosity for the interpopulation cross between C0 and C12, whereas the 54 neutral loci did not.

Effective population size

Empirical estimates of N_e and 95% confidence intervals for all loci, neutral loci and nonneutral loci are shown in Table 7. The latter two categories were based on Waples' (1989a) test results (Table 5). The three different estimators of $\hat{f}$ gave similar results. For all loci, estimates of N_e ranged approximately between N(12) and $2N$–1. For neutral loci, N_e was closer to $2N$–1. Nonneutral loci experienced a large reduction in N_e in comparison to neutral loci.

The estimator of Krimbas and Tsakas (1971) uses only the observed initial frequency of an allele to estimate the population's frequency, while the other two estimators use allele frequency information from initial and later samples. This may imply that the latter estimators are to be preferred (E. Pollak, personal communication). Estimates of N_e were consistently smaller using Krimbas and Tsakas' $\hat{f}$ when compared to Nei and Tajima's (1981) and Pollak's (1983) $\hat{f}$.

Inclusion of low-frequency alleles in these estimates will downwardly bias $\hat{f}$, which in turn upwardly biases the estimate of N_e (R. Waples, personal communication). Estimates obtained using the frequency of the most common allele at C0 for each locus and lumping all other alleles together yielded estimates closer to N_e=N=12 and N_e=$2N$–1=23 for all loci and neutral loci, respectively, while estimates for nonneutral loci were about 0.5 N (data not shown). The estimates of N_e for BSCB1(R) may be more accurate than for BSSS(R) because BSSS(R)C0 had already undergone some drift before sampling, as discussed previously. This may be the reason that the empirical estimate of $\bar{f}$ was generally smaller in BSSS(R).

Discussion

The amount of genetic variation remaining in the BSSS(R) and BSCB1(R) RRS breeding program relative to the C0 populations has practical implications. Labate et al. (1997) looked at gene diversity within BSSS(R) and BSCB1(R) populations and found substantial decreases between C0 and C12, with an almost tenfold increase in genetic distance between the two C12 populations relative to progenitors. They also measured total gene diversity of hypothetical crosses between various BSSS(R) and BSCB1(R) populations (as in Table 6 of this paper), a pragmatic measure in a breeding program such as this. The estimators used in Table 4 are different in that they measured gene diversity by using the mean allele frequency of all alleles at a locus for the total gene pool. This is a purely theoretical approach because in practice the germplasm for the two populations would not be pooled or mixed. The total expected heterozygosity was reduced 15% between the pooled C0 and C12 populations. The observation that total expected heterozygosity in C12 was larger than the expected heterozygosity within subpopulations indicates that the two populations are becoming differentiated in terms of the identities of alleles that are reaching high frequencies. The between-population component of genetic variation increased substantially between P and C12. In theory, a consequence of RRS is the increase of this between-population component by fixation of complementary alleles in the two populations through selection. The patterns of change in genetic diversity are consistent with the theoretical expectations of RRS but raise the obvious question – how much has selection influenced these changes?

Specific allele frequency changes have yielded some insight into the answer to this question. About 30% of the loci were characterized by an estimated frequency of the most common allele as being greater than 0.90 by C12. At equilibrium, virtually all loci will contain an allele at fixation in each of the two populations. Are some of the loci approaching this equilibrium condition more rapidly than would be expected solely by drift of neutral alleles? According to results from Waples' (1989a) test, about 17% of all loci surveyed within a population rejected the null hypothesis that genetic drift was solely responsible for their allele frequency changes between C0 and C12. These loci were not limited to particular chromosomes or regions but seemed to be spread rather evenly throughout the genome. They fit a pattern of fixation of complementary alleles between the two populations because none of the alleles were shared. The mean expected heterozygosity of the interpopulation cross increased for the nonneutral loci, implying that an intralocus mechanism could be responsible to some extent for increasing interpopulation hybrid performance. These results must be interpreted with caution because we assume that we are identifying hitchhiking loci rather than the selected loci *per se*. Also, it is possible that with a more powerful test many more loci will be identified as rejecting the null hypothesis of drift in the two populations and that a larger fraction will be shared. Of course, natural selection cannot be ruled out as influencing allele frequency changes in the populations.

Waples (1989a) states that his test is perhaps not the most powerful one available when a specific hypothesis other than drift is to be tested. We plan on collecting data from at least one intermediate time point to apply a linear model that tests the hypothesis of directional selection (Schaffer et al. 1977; Wilson 1980) and also to repeat Waples' test with multiple time points.

Estimates of effective population sizes based on temporal changes in allele frequency between C0 and C12 for both BSSS(R) and BSCB1(R) fell within the range predicted by the breeding method; that is, the harmonic mean of the number of lines selected at each cycle, N, or approximately twice that number. Directional selection

seems to deflate effective population size. We hypothesize that in unselected populations N_e would equal $2N$–1; selected populations will not be likely to reach that value because of genetic hitchhiking. It is worth examining the breeding method in more detail to understand the origin of the predicted N_e. There were not 10 or 20 selected individuals at the completion of each cycle but 10 or 20 selected S_1 lines. A set of S_1 lines represents the progeny from a self-fertilized individual. In practice, when selecting 10 S_1 lines to create the population representing the next cycle, seed from 90 ears is bulked. This includes 1 ear from each cross of all 90 reciprocal diallel crosses. If each S_1 line can be thought of as a sample of the selecting individual's gamete pool, then more extensive gametic sampling is taking place than would by selection of individual plants with no selfing. This would reduce the variance in drift resulting from meiosis. This extensive sampling could also average out environmental variation leading to variance in the fertility of particular plants.

The results from the molecular analyses correspond well with interpretations made of phenotypic data on grain yield in BSSS(R) and BSCB1(R) populations. Keeratinijakal and Lamkey (1993a) reported that 11 cycles of RRS increased grain yield of the interpopulation cross (BSSS(R)Cn×BSCB1(R)Cn) by an average of 7% per cycle. Inbreeding depression for the interpopulation cross, as measured by selfing their F_1, also increased with cycles of selection. They concluded that the increase in inbreeding depression was due to selection for complementary alleles at loci in each of the populations. This would result in an increase in heterozygosity of the interpopulation cross with cycles of selection because a different allele is being fixed at a given locus in each population.

The genetic distance between the BSSS(R)C11 and BSCB1(R)C11 populations would also increase if different alleles were being fixed at the same loci in the two populations. Hanson (1987) proposed using dominance-associated distance (D_d) as a relative measure of genetic distance. D_d is related to specific combining ability effects obtained from a diallel-mating scheme among populations. Distances between populations based on dominance-associated gene effects increased 3.5 times from BSSS(R)C0 vs. BSCB1(R)C0 to BSSS(R)C11 vs. BSCB1(R)C11 (Keeratinijakal and Lamkey 1993b). Although not as great as the tenfold increase in genetic distance calculated with molecular marker data reported by Labate et al. (1997), the results do suggest a correspondence between the distance estimates based on molecular markers and grain yield. Selection for complementary alleles at each locus is possible under both dominance and overdominance models of gene action. Changes in patterns of D_d for grain yield among populations suggest that overdominance was not important and selection was for loci with partial to complete dominance (Keeratinijakal and Lamkey 1993b).

Holthaus and Lamkey (1995) reported on experiments designed to estimate additive and dominance genetic variances in BSSS. They found that 11 cycles of RRS in BSSS decreased additive variance by 23% and dominance variance by 76%. The loss in total genetic variance (additive and dominance) was 50%. These trends in genetic variance components parallel genetic diversity loss measured with DNA markers (Table 4). The decrease in dominance variance for grain yield may be an indication that heterozygosity within BSSS has substantially decreased with selection as demonstrated by the molecular marker data.

It is well-known that grain yield in maize shows substantial inbreeding depression (Hallauer and Miranda 1988, chapter 9). Grain yield also seems to be controlled by a large number of loci distributed throughout the genome. The relationship between molecular marker data and grain yield indicates that grain yield may be a sensitive indicator of the heterozygosity level of a population. Changes in heterozygosity due to inbreeding (as detected by grain yield), however, are confounded with changes resulting from selection and drift. For a trait as complex as grain yield, it will be a challenge to relate genomic changes detected by molecular markers to changes in phenotype.

Acknowledgments We thank Dr. E. Pollak for comments on the manuscript. This study was supported in part by a grant from Pioneer Hi-Bred International, Inc, to KRL and ML. JAL is funded by the USDA-ARS Postdoctoral Research Associate Program. This work is a joint contribution of the Corn Pest and Crop Genetics Research Unit, USDA-ARS, Department of Agronomy, Iowa State University and Journal Paper No. J-17302 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, 50011–1010, project Nos. 3082 and 3134, and supported by Hatch Act and State of Iowa Funds.

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